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ORIGINAL ARTICLE

Dietary Intervention in Infancy and Later Signs of Beta-Cell Autoimmunity

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ABSTRACT

BACKGROUND

Early exposure to complex dietary proteins may increase the risk of beta-cell autoimmunity and type 1 diabetes in children with genetic susceptibility. We tested the hypothesis that supplementing breast milk with highly hydrolyzed milk formula would decrease the cumulative incidence of diabetes-associated autoantibodies in such children.

METHODS

In this double-blind, randomized trial, we assigned 230 infants with HLA-conferred susceptibility to type 1 diabetes and at least one family member with type 1 diabetes to receive either a casein hydrolysate formula or a conventional, cow's-milk–based formula (control) whenever breast milk was not available during the first 6 to 8 months of life. Autoantibodies to insulin, glutamic acid decarboxylase (GAD), the insulinoma-associated 2 molecule (IA-2), and zinc transporter 8 were analyzed with the use of radiobinding assays, and islet-cell antibodies were analyzed with the use of immunofluorescence, during a median observation period of 10 years (mean, 7.5). The children were monitored for incident type 1 diabetes until they were 10 years of age.

RESULTS

The unadjusted hazard ratio for positivity for one or more autoantibodies in the casein hydrolysate group, as compared with the control group, was 0.54 (95% confidence interval [CI], 0.29 to 0.95), and the hazard ratio adjusted for an observed difference in the duration of exposure to the study formula was 0.51 (95% CI, 0.28 to 0.91). The unadjusted hazard ratio for positivity for two or more autoantibodies was 0.52 (95% CI, 0.21 to 1.17), and the adjusted hazard ratio was 0.47 (95% CI, 0.19 to 1.07). The rate of reported adverse events was similar in the two groups.

CONCLUSIONS

Dietary intervention during infancy appears to have a long-lasting effect on markers of beta-cell autoimmunity — markers that may reflect an autoimmune process leading to type 1 diabetes. (Funded by the European Commission and others; ClinicalTrials.gov number, NCT00570102.)

Type 1 diabetes is defined by the loss of insulin-producing beta cells in the pancreatic islets in genetically susceptible persons. Overt diabetes is preceded by an asymptomatic period of highly variable duration[1] during which diabetes-associated autoantibodies appear in the peripheral circulation as markers of emerging beta-cell autoimmunity. Five disease-related autoantibodies predict the clinical manifestation of type 1 diabetes: islet-cell antibodies; insulin autoantibodies; and autoantibodies to glutamic acid decarboxylase (GAD), the tyrosine phosphatase-related insulinoma-associated 2 molecule (IA-2), and zinc transporter 8 (ZnT8).[2,3] Positivity for two or more antibodies signals a risk of 50 to 100% for the development of type 1 diabetes over the course of 5 to 10 years.[4]

Accumulating evidence suggests that beta-cell autoimmunity may be induced early in life.[5,6] The incidence of type 1 diabetes is rising faster than it had previously among children, particularly among children younger than 5 years of age.[7,8] Food content in early childhood may modify the risk of type 1 diabetes later in life. A short duration of breast-feeding and early exposure to complex dietary proteins have been implicated as risk factors for advanced beta-cell autoimmunity or clinical type 1 diabetes.[9,10] Early nutritional intervention may help to prevent type 1 diabetes and has been reported to be successful in experimental models of autoimmune diabetes, although the data are not consistent.[11-14] Our preliminary data indicated that among children at increased risk for type 1 diabetes, weaning to a highly hydrolyzed formula decreased the cumulative incidence of islet-cell antibodies and the cumulative incidence of at least one autoantibody during a mean observation period of 4.7 years.[15] In this article, we report findings from the pilot study of the Trial to Reduce IDDM in the Genetically at Risk (TRIGR), which documents the rates of beta-cell autoimmunity and progression to clinical diabetes in children up to 10 years of age.

METHODS

STUDY DESIGN

We conducted a randomized, double-blind study at 15 hospitals in Finland. Newborn infants who had a first-degree relative with type 1 diabetes were recruited between February 1995 and November 1997. Written informed consent was obtained from the mothers before enrollment. The protocol that was initially approved by the ethics committee at each participating hospital called for observation of the children until they reached 2 years of age; it was subsequently modified to allow for observation of the children until they reached 10 years of age. The protocol, including the statistical analysis plan, is available with the full text of this article at NEJM.org. An agreement that the results would remain confidential until publication was in force between the members of the TRIGR Study Group and Mead Johnson Nutrition, which provided the study formulas. Mead Johnson Nutrition had no role in the design of the study, the accrual or analysis of the data, or the preparation of the manuscript. The first author wrote the initial draft of the manuscript; all the authors contributed to the final version of the manuscript and vouch for the accuracy and completeness of the reported data, as well as the fidelity of the report to the trial protocol.

STUDY PARTICIPANTS

Over the course of 34 months, we identified 520 newborn infants for possible inclusion in the study. A total of 45 did not meet the inclusion criteria, mainly owing to prematurity (gestational age of <36 weeks) or the unavailability of a cord-blood sample for HLA genotyping. Altogether, 475 newborn infants received a study code at birth, and HLA genotypes were obtained for 470, usually within 1 week after birth; 230 newborn infants with an eligible HLA risk genotype (49% of the infants for whom HLA genotypes were obtained) continued in the intervention study (Fig. 1), of whom 131 (57%) were boys. A total of 85 infants (37%) had a mother with type 1 diabetes, 100 (43%) had an affected father, 35 (15%) had an affected sibling, and 10 (4%) had more than one affected first-degree relative.

AUTOANTIBODY DATA

Blood samples were obtained (after application of analgesic cream) at the follow-up visits when the children were 3, 6, 9, 12, 18, and 24 months of age and thereafter when they were 3, 5, 7, and 10 years of age. Serum samples were stored at –70°C until they could be analyzed. Autoantibody data were obtained from 208 children (90%) who had at least one follow-up sample available. The

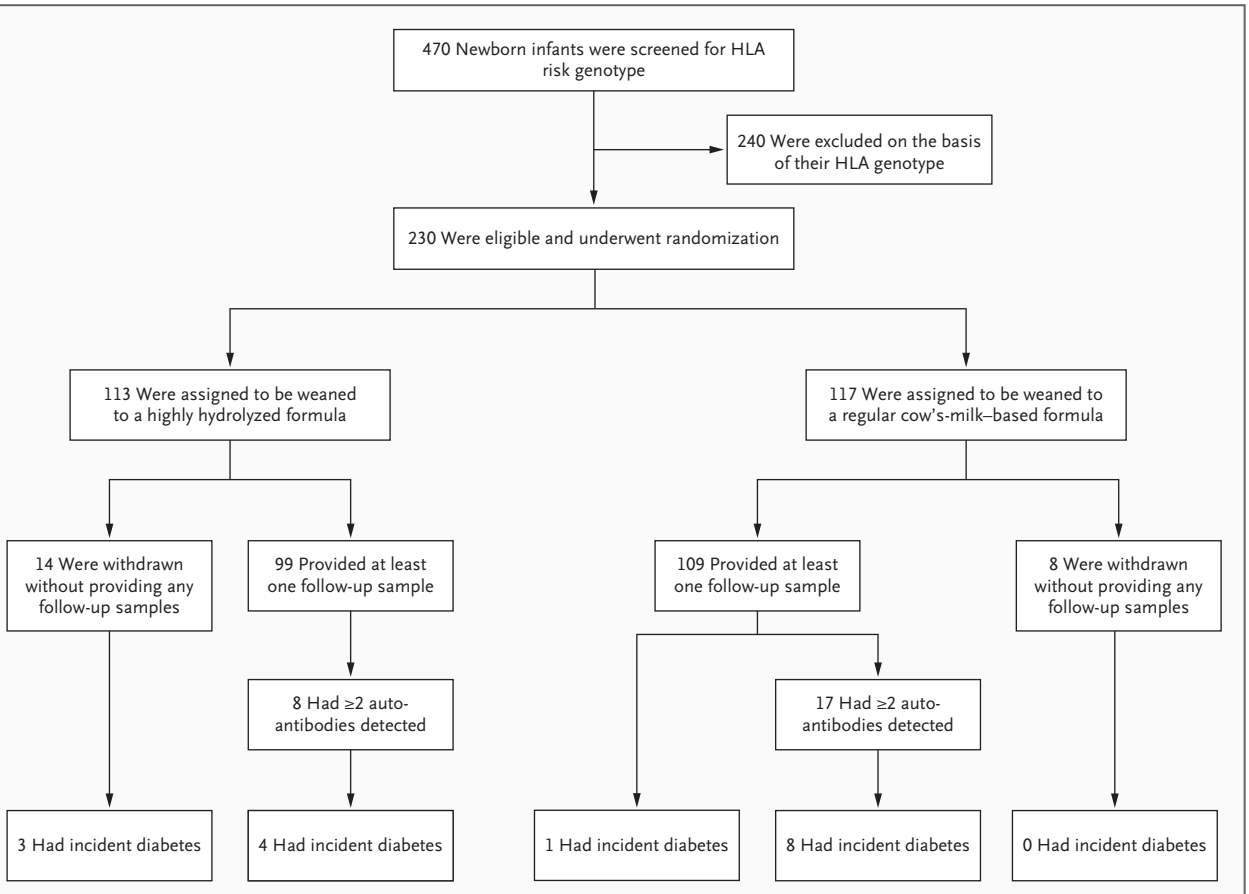


Figure 1. Screening, Randomization, and Follow-up.

mean follow-up time for the detection of autoantibodies was 7.5 years (median, 10 years; range, 3 months to 10 years).

DIETARY INTERVENTION

Infants were randomly assigned after birth to receive either the intervention formula or a control formula whenever breast milk was not available. The intervention formula was an extensively hydrolyzed casein-based formula (Nutramigen, Mead Johnson Nutrition); the control formula, which was produced specifically for this study, was composed of 80% intact milk protein (Enfamil) and 20% hydrolyzed milk protein and was formulated so that the taste and smell would be indistinguishable from those of the intervention formula. Study formulas were prepared and coded with the use of four colors by Mead Johnson Nutrition, which held the codes. Newborn infants requiring supplemental feeding before randomization (e.g., infants born at night or on weekends) received banked breast milk or Nutramigen. The codes, which were broken, as specified by the protocol, when the youngest child in the study completed the intervention, revealed that 113 infants had been randomly assigned to the casein hydrolysate group and 117 to the control group. There were no differences in the distribution of HLA genotypes or of affected family members between the two groups (Table 1 in the Supplementary Appendix, available at NEJM.org).

Breast-feeding was practiced at the discretion of the participating mothers, and maternal diets were unmodified. Breast-feeding was encouraged and exceeded national averages in both study groups. The dietary intervention period lasted until the infant was at least 6 months of age. If the mother chose to breast-feed exclusively until the child was 6 months of age, the opportunity to use the study formula was extended for 2 months, until the child was 8 months of age. Parents were told not to feed the children any commercial

baby foods and other foods containing bovine protein during the intervention period. Adherence to the protocol was monitored by means of regular family interviews and by analysis of cow's-milk antibodies in serum samples.

HLA GENOTYPING

An analysis of cord-blood HLA-DQB1 genotype was performed to identify selected alleles (DQB1*02, *0301, *0302, and *0602/3) that are known to be significantly associated with susceptibility to or protection against type 1 diabetes.[16] In DQB1*02-positive infants, the DQA1 allele was also analyzed. The genotyping technique is based on solution hybridization with lanthanide-labeled oligonucleotide probes detected with the use of time-resolved fluorometry.[17] Infants were eligible for the trial if they carried the high-risk HLA-DQB1*02/DQB1*0302 genotype (51 infants [22%]), the moderate-risk DQB1*0302/x genotype (x≠DQB1*0301, *0602, or *0603) (92 infants [40%]), or the HLA-DQA1*05-DQB1*02(DR3)/y genotype (y≠DQB1*0301, *0602, or *0603) (87 infants [38%]), which, although it confers an increased risk of disease, confers a risk lower than that with the other genotypes.

DETECTION OF BETA-CELL AUTOIMMUNITY

Islet-cell antibodies were detected with the use of indirect immunofluorescence, whereas the other four autoantibodies were quantified with the use of specific radiobinding assays.[18] We used cutoff limits for positivity of 2.5 Juvenile Diabetes Foundation (JDF) units for islet-cell antibodies, 3.48 relative units (RU) for insulin autoantibodies, 5.36 RU for GAD autoantibodies, 0.43 RU for IA-2 autoantibodies, and 0.61 RU for ZnT8 autoantibodies. The disease sensitivity and specificity of the assay for islet-cell antibodies were 100% and 98%, respectively, in the fourth round of the international workshops on standardization of the ICA assay. The disease sensitivity and specificity of the assay for insulin autoantibodies were 58% and 100%, respectively, in the 2005 Diabetes Antibody Standardization Program (DASP) workshop. The corresponding characteristics of the assay for GAD autoantibodies were 82% and 96%, and those of the assay for IA-2 autoantibodies were 72% and 100%. The assay for ZnT8 autoantibodies had a sensitivity of 50% and a specificity of 100% in the 2009 DASP workshop. Maternal antibodies that were transferred through the placenta, as verified by detectable autoantibodies in a maternal blood sample, were not included in the statistical analysis.

PROGRESSION TO TYPE 1 DIABETES

Progression to type 1 diabetes was recorded, although it was not the primary end point of the study. The information about incident diabetes was obtained mainly from the pediatric clinics at which the children were followed. In addition, as a secondary source, the incidence of diabetes in the entire cohort was confirmed by means of information obtained from a national drug reimbursement registry that includes data from virtually the entire population of Finland.[19]

STATISTICAL ANALYSIS

The differences between the two groups with respect to the autoantibody titers and the duration of exposure to the study formula were assessed with the use of the Mann–Whitney U test. In the case of a child who was positive for antibodies, seroconversion was considered to have occurred sometime between the last time a negative test was obtained and the time of the first positive measurement (an interval-censored survival time). If a child had only negative measurements, no seroconversion was assumed to have occurred before the last measurement (a right-censored survival time). A nonparametric survival analysis was performed with the use of the self-consistency algorithm of Turnbull[20] to obtain maximum-likelihood estimates of the survival functions of seroconversion to positivity for diabetes-predictive autoantibodies during the follow-up period. The hazard ratios associated with exposure to the formula were estimated with the use of flexible parametric models, in which restricted cubic splines are used to smooth the baseline log cumulative hazard.[21]

Cox proportional-hazards regression was used to analyze the association between the intervention and the risk of clinical type 1 diabetes. The analyses were adjusted for the duration of exposure to the formula and for the child's age at the time the formula was introduced. Both of these variables were treated as time-dependent covariates and were used as continuous variables. Whenever both were included in the model, the interaction between duration and age was allowed. The likelihood functions of the regression models were based on interval-censored survival times and were maximized with the use of the Broyden–Fletcher–Goldfarb–Shanno (BFGS) algo-

rithm of the "optim" function in the R statistical package.[22] The interval package in R was used to calculate the nonparametric maximum-likelihood estimates of the survival functions.

The intention-to-treat principle was used for the analyses of seroconversion to autoantibody positivity and progression to type 1 diabetes. The analysis of progression to type 1 diabetes was also performed according to treatment received (per-protocol analysis). All tests were two-tailed; P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

STUDY INTERVENTION

The median age of the infants at the time the study formula was introduced was 2.6 months in the casein hydrolysate group and 1.1 months in the control group (P=0.03). The median age at the time the intervention was completed was 7.4 months in the casein hydrolysate group and 6.4 months in the control group (P=0.15). The median duration of study-formula feeding was 3.3 months in the casein hydrolysate group and 4.9 months in the control group (P=0.05).

BETA-CELL AUTOIMMUNITY

A total of 208 children (90%) — 99 in the casein hydrolysate group and 109 in the control group — provided at least one blood sample during the follow-up period for determination of diabetes-associated autoantibodies. At least one autoantibody developed in 17 of the children in the casein hydrolysate group (17%) and 33 in the control group (30%). Eight children in the casein hydrolysate group (8%), as compared with 17 in the control group (16%), tested positive for two or more autoantibodies; data from each of these children on sex, genotype, and the autoantibodies that developed, as well as the age of the children at the time they developed, are provided in Table 2 in the Supplementary Appendix. Insulin autoantibodies were the first or among the first antibodies that appeared in children in whom multiple autoantibodies were already detectable in the first positive sample (15 of the 25 children with multiple autoantibodies [60%]). Islet-cell antibodies were also the first or among the first antibodies that appeared (15 of 25 children [60%]), whereas 5 children (20%) tested positive for IA-2 autoantibodies and only 1 child (4%) tested positive for ZnT8 autoantibodies in the first autoantibody-positive sample.

The earliest age at which autoantibodies were detected was 6 months (Patient 26 in Table 2 in the Supplementary Appendix), and the latest seroconversion was observed when the child was 10 years of age. Among the children who tested positive for autoantibodies, there were no significant between-group differences in the initial or maximal autoantibody titers (data not shown). The cumulative incidences of islet-cell antibodies, insulin autoantibodies, and IA-2 autoantibodies in the casein hydrolysate group and in the control group, according to the age of the children at the time the autoantibodies were detected, are shown as nonparametric maximum-likelihood estimates in Figure 2. The cumulative incidences of at least one autoantibody and of at least two autoantibodies in the two groups are shown in Figure 3. Between-group comparisons of the cumulative incidence of GAD autoantibodies and ZnT8 autoantibodies are provided in Figure 1 in the Supplementary Appendix.

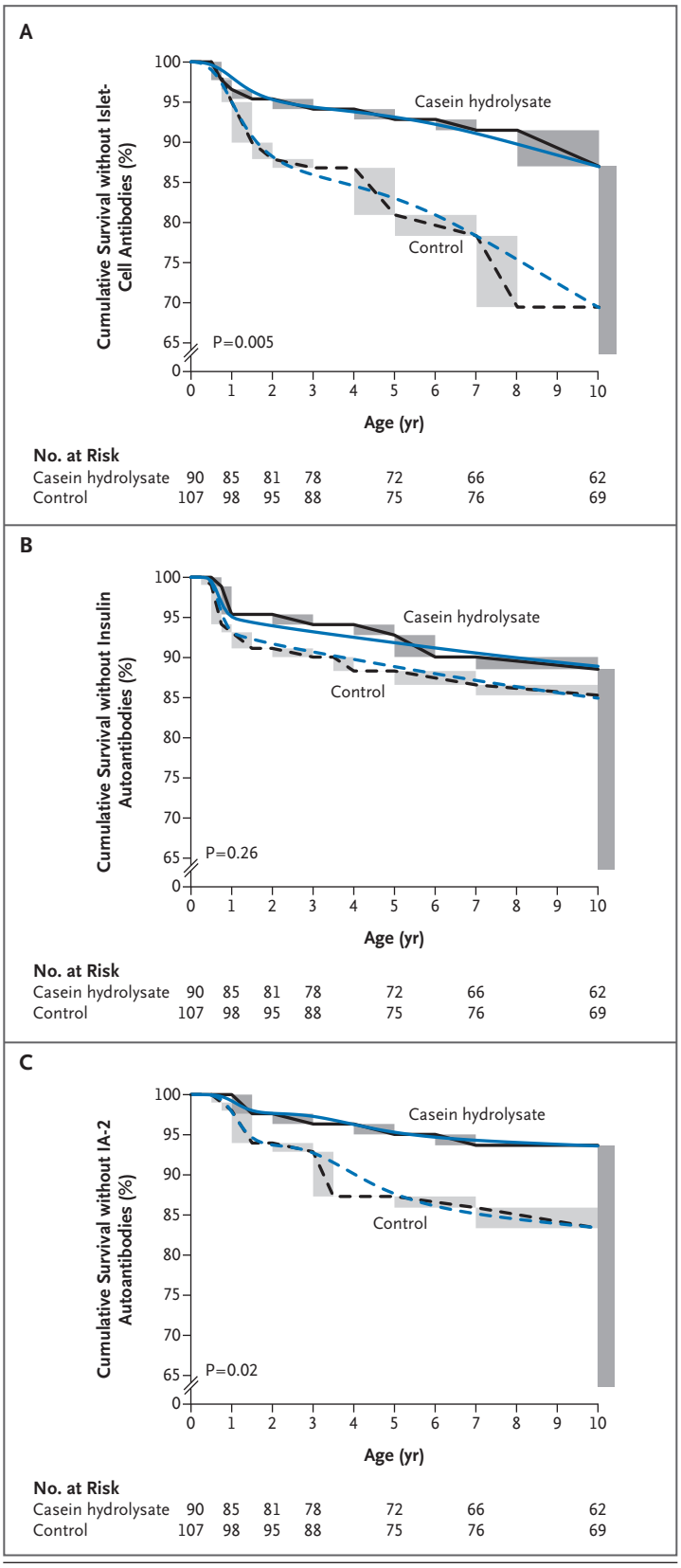
The results of the analysis of hazard ratios are shown in Table 1. Feeding with the casein hydrolysate formula was associated with a decrease in the risk of seroconversion to positivity for islet-cell antibodies, IA-2 autoantibodies, and at least one autoantibody (one or more of islet-cell antibodies, insulin autoantibodies, GAD autoantibodies, IA-2 autoantibodies, and ZnT8 autoantibodies). As noted above, there was a difference between the two groups in the duration of exposure to the study formula. After adjustment for this variable, a significant protective effect of the intervention against positivity for islet-cell antibodies, IA-2 autoantibodies, and at least one autoantibody was noted, whereas there was a trend for a protective effect against positivity for at least two autoantibodies (Table 1). Further adjustments for the age at introduction of the study formula or for both age at introduction and duration of exposure to the study formula resulted in no changes or only minor changes in the hazard ratios (Table 3 in the Supplementary Appendix).

PROGRESSION TO OVERT DIABETES

Type 1 diabetes developed in 16 children (7%) by the time they were 10 years of age — 7 of the 113 children in the casein hydrolysate group (6%) and 9 of the 117 children in the control group (8%). The mean age at diagnosis was 4.8 years (range,

Figure 2. Cumulative Incidence of Islet-Cell Antibodies, Insulin Autoantibodies, and Autoantibodies to the Insulinoma-Associated 2 Molecule (IA-2) in the Two Study Groups.

The cumulative incidence of islet-cell antibodies (Panel A), insulin autoantibodies (Panel B), and IA-2 autoantibodies (Panel C) in the casein hydrolysate group and in the control group, according to the age of the children at the time the autoantibodies were detected, is shown, with the use of nonparametric maximum-likelihood estimates (black) and fitted curves (blue) of the regression models. For the nonparametric maximum-likelihood estimates, gray rectangles show the intervals within which any curve would maximize the likelihood. The P values are derived from analyses adjusted for the duration of exposure to the study formula.



0.8 to 9.6). The risk for type 1 diabetes was not significantly associated with the feeding intervention (hazard ratio with casein hydrolysate, 0.80; 95% confidence interval [CI], 0.30 to 2.14). The hazard ratio adjusted for the difference in the duration of exposure to the study formula was 0.48 (95% CI, 0.14 to 1.61). Three children in whom overt type 1 diabetes developed dropped out of the study before the age of 3 months — two within 2 to 4 days after birth and the third just before the 3-month follow-up visit. All three children had been randomly assigned to the casein hydrolysate group, but none had received any study formula. Their diagnostic information was obtained from the Finnish Social Insurance Registry. Accordingly, the number of children in the per-protocol cohort (i.e., the cohort defined according to the treatment received) who progressed to type 1 diabetes was four in the casein hydrolysate group (4%) and nine in the control group (8%). In the per-protocol cohort, the hazard ratio for type 1 diabetes with casein hydrolysate was 0.40 (95% CI, 0.11 to 1.51). Of the 13 children in the per-protocol cohort in whom diabetes developed, all but 1 (Patient 26 in Table 2 in the Supplementary Appendix) had samples that tested positive for multiple autoantibodies in the preclinical period. The child who did not test positive in the preclinical period was positive for insulin autoantibodies in a sample obtained at the age of 6 months (the last available sample for that infant) — 14 months before the clinical presentation.

ADVERSE EVENTS

The rate of reported adverse events was similar in the two groups. Details of the adverse events that

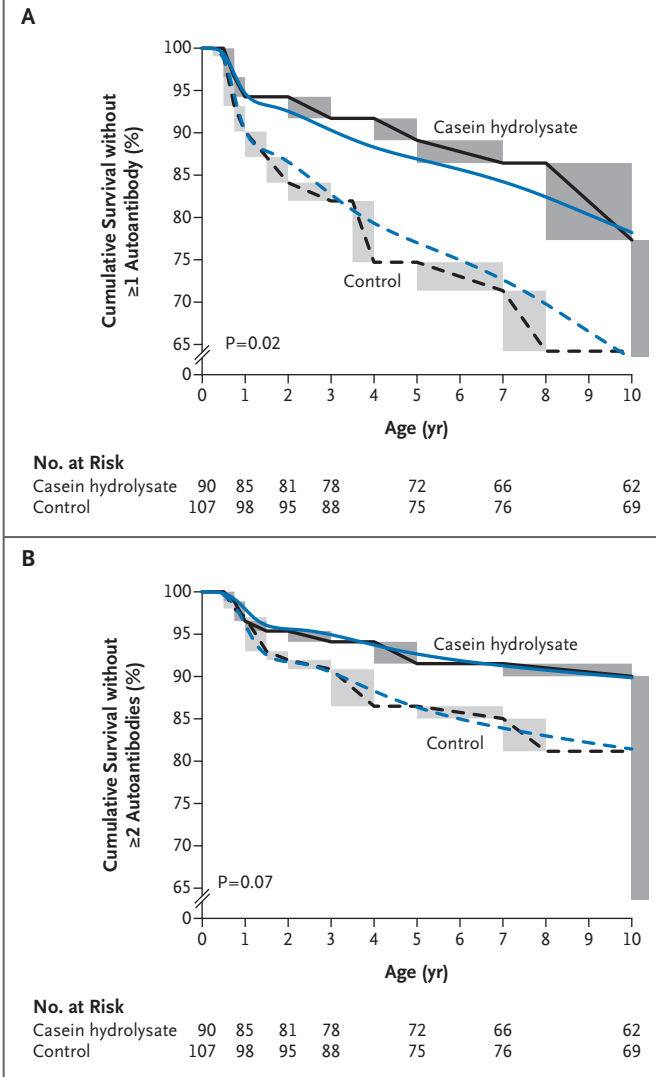


Figure 3. Cumulative Incidences of at Least One Autoantibody and of at Least Two Autoantibodies.

The cumulative incidences of at least one autoantibody (Panel A) and at least two autoantibodies (Panel B) in the casein hydrolysate group and in the control group, according to the age of the children at the time the autoantibodies were detected, are shown, with the use of nonparametric maximum-likelihood estimates (black) and fitted curves (blue) of the regression models. For the nonparametric maximum-likelihood estimates, gray rectangles show the intervals within which any curve would maximize the likelihood. The P values are derived from analyses adjusted for the duration of exposure to the study formula.

were reported are provided, according to study group, in Table 4 in the Supplementary Appendix.

DISCUSSION

Our study showed that among children with an HLA genotype conferring increased risk for type 1 diabetes and a first-degree relative with type 1 diabetes, weaning to a highly hydrolyzed formula during infancy was associated with fewer signs of beta-cell autoimmunity up to 10 years of age. Our study was not powered for any definitive conclusion concerning progression to overt type 1 diabetes, but a larger TRIGR study now ongoing in 15 countries on three continents is designed with adequate power to address that issue.[23]

Preliminary data from our trial, case–control studies,[24] and observations from studies in nonobese diabetic mice[11] and BioBreeding rats[12] suggest that complex foreign-protein diets during weaning may play a deleterious role in the process leading to autoimmune diabetes. In the control group in our study, the cumulative incidence of multiple autoantibodies by the age of 6 years was 12% (derived from data in Table 2 in the Supplementary Appendix), and the cumulative incidence of type 1 diabetes by the age of 10 years was 8%.

Our data suggest that weaning to a highly hydrolyzed formula, as compared with a cow's-milk–based formula, was associated with a decreased risk of positivity for at least one diabetes-associated autoantibody, as reflected by an unadjusted hazard ratio with the highly hydrolyzed formula of 0.54 and by a hazard ratio after adjustment for the difference in the duration of exposure to the study formula of 0.51. The corresponding hazard ratios for multiple (≥2) autoantibodies were 0.52 and 0.47. The observed decrease in the risk of positivity for GAD autoantibodies was smaller than that for the other autoantibodies, a finding that is not surprising given that, in contrast to the other autoantibodies, GAD autoantibodies have been shown to be associated with a low risk for progression to type 1 diabetes among young children with HLA-defined disease susceptibility.[25] The mechanisms by which hydrolyzed formula decreases the risk of diabetes-predictive autoantibodies are not known. We speculate that potential mechanisms may involve reduced gut permeability, induction of the maturation of regulatory T cells in the gut-associated lymphoid tissue, modification of the gut microflora, or some combination of these. A recent study showed that highly hydrolyzed formula was associated with a decrease in autoimmune diabetes in the disease-prone BioBreeding rat, in association with improved integrity of the intestinal barrier and production of regulatory cytokines, as well as beneficial changes in gut microflora.[26]

Table 1. Hazard Ratios with Highly Hydrolyzed Infant Formula, as Compared with Conventional Cow's-Milk–Based Formula, for Seroconversion to Positivity for Autoantibodies Predictive of Type 1 Diabetes.*

Autoantibodies	No. Who Underwent Seroconversion	Hazard Ratio with Highly Hydrolyzed Formula (95% CI)	P Value	Adjusted Hazard Ratio with Highly Hydrolyzed Formula (95% CI)†	P Value
Islet-cell antibodies	37	0.38 (0.18–0.77)	0.006	0.37 (0.17–0.75)	0.005
Insulin autoantibodies	23	0.72 (0.30–1.64)	0.45	0.61 (0.25–1.42)	0.26
GAD autoantibodies	23	0.87 (0.37–1.97)	0.74	0.80 (0.34–1.85)	0.61
IA-2 autoantibodies	20	0.36 (0.12–0.94)	0.04	0.32 (0.10–0.83)	0.02
ZnT8 autoantibodies	14	0.61 (0.19–1.77)	0.37	0.61 (0.19–1.79)	0.37
≥1 Antibody	50	0.54 (0.29–0.95)	0.03	0.51 (0.28–0.91)	0.02
≥2 Antibodies	25	0.52 (0.21–1.17)	0.12	0.47 (0.19–1.07)	0.07

* A total of 208 children (90% of the 230 participants who continued in the pilot trial after HLA genotyping) had a minimum of one serum sample available for autoantibody testing: 99 in the group that was randomly assigned to receive the hydrolyzed formula and 109 in the group that received the cow's-milk–based formula. GAD denotes glutamic acid decarboxylase, IA-2 insulinoma-associated 2 molecule, and ZnT8 zinc transporter 8.

† Adjusted hazard ratios were adjusted for the duration of exposure to the study formula.

Our study was not designed to test the effect of breast-feeding, since randomly assigning infants to breast-feeding or formula feeding would not be ethical. Some prospective studies assessing the associations between infant feeding patterns and the development of beta-cell autoimmunity in children who are at genetic risk for type 1 diabetes have not detected any associations between the duration of either exclusive or total breast-feeding and beta-cell autoimmunity.[27-29] However, a single-cohort study involving children in the general population showed that a short duration of breast-feeding was related to an increased risk of positivity for GAD autoantibodies, insulin autoantibodies, or both.[30]

Our results indicate that a preventive dietary intervention aimed at decreasing the risk of type 1 diabetes may be feasible. Such an intervention would need to be initiated early in life, since the first signs of beta-cell autoimmunity may appear before a child reaches the age of 3 months.[6] If such an intervention is shown to be effective and safe in high-risk children, a next step might be to expand the intervention to a wider infant population, since 83 to 98% of children with newly diagnosed type 1 diabetes are from the general population.[31] Nutritional intervention during infancy, such as that provided in this study, may be an attractive strategy, since it could be implemented relatively easily as a public health measure.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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